MUCUS SECRETION IN THE TRACHEA.

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IN the trachea and bronchi there are two distinct sets of glands producing mucus. As shown in Fig. 1, goblet-cells are fairly numerous in the tracheal mucosa of the cat. Usually they give a typical red coloration with mucicarmine. Empty, collapsed goblet-cells, not giving this reaction, may often be observed. These cells are absent over the posterior tracheal fold.

There are found, in addition, acini of gland-cells lying in the submucosa. These cell-groups are serous, mucous or mixed in type. The submucosal glands, in contrast with the goblet-cells, have been shown to be innervated (Larsell, 1923).

These present experiments have had as their object the determination of the conditions in which mucus is discharged from these two groups of glands, and the observation of the histological changes involved in their activity.

EXPERIMENTAL.

Cats have been used almost exclusively for these investigations. The various methods employed will be described under the different experiments.

Effects of Stimulation of Nerves.

A straight tracheal cannula was inserted into a cat, anæsthetized by dial or chloralose, or decerebrated. One vagus nerve was dissected in the neck, tied and cut. During a control period of 3 hours it was observed that no obvious mucus collected in the cannula. The peripheral end of the vagus was then stimulated faradically, each stimulus lasting 1 1/5 seconds, with an intervening rest period of 1 second. At the end of a few minutes mucus secretion began to appear in the cannula, being pushed up from the trachea—presumably by the cilia. The secretion of mucus continued throughout the duration of the stimulus, and considerable quantities had to be evacuated from the cannula to prevent asphyxia. A method for measuring the amount of secretion obtained from a given length of trachea during a given time was devised. The technique of preparing the trachea and the methods of recording are fully described elsewhere (Florey and Wells, 1931). Briefly, a length of trachea is enclosed between two cannulæ, one of which is attached to a horizontal

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graduated pipette by rubber tubing. The trachea is filled with oil, which extends to the beginning of the pipette. Any alteration of the volume of the enclosed trachea is recorded by an alteration of the reading of the oil in the pipette. This alteration can be due either to contraction of the tracheal muscle or to secretion of fluid into the tracheal lumen. Atropine will abolish the former when due to nervous influences or parasympathicomimetic drugs, so that the administration of this drug at the end of stimulation enables a separation to be made between secretion and contraction effects.

Preliminary experiments showed that when pure olive oil was used, the reading of the oil in the pipette usually remained remarkably constant during long periods. The administration of atropine or adrenalin did not alter this significantly. Disturbances such as swallowing had little effect.

The following are figures taken from an experiment in which the recurrent laryngeal nerves had been tied and cut low in the neck, and the peripheral ends subsequently stimulated for 1 minute alternately during the times given:

Time.	Reading of oil.	Increase. (1 div. = 0·2 c.c.)
12.44 p.m.	3·79	..
Stimulus.		
1.4	4·27	0·48
1.24	4·71	0·44
Left till 2.30. Level of oil brought back (as 5·00 was the end of scale).		
2.32	3·58	..
Stimulus.		
2.52	3·98	0·40
0·75 c.c. 1 per cent. atropine given. Stimulated again.		
3.12	4·02	0·04

By this means it was possible to measure the quantity of secretion from a given length of trachea over a given period of time, and to show the inhibition of secretion by atropine.

A further modification of this procedure was made by recording photographically the movements of the oil by means of the apparatus described elsewhere (Florey and Wells, 1931).

Fig. 6 is such a record of the amount of secretion obtained by stimulating for 1 minute the peripheral ends of the cut vagi alternately during 15 minutes. After the cessation of the stimuli atropine was given intravenously, which did not alter the oil level. A stimulation for a further period of 15 minutes produced no rise. Examination of the trachea post-mortem showed a healthy mucosa with a collection of clear mucoid fluid at the upper end. These experiments clearly show that mucus is secreted by the trachea when the vagus and inferior laryngeal nerves are stimulated, the effect being abolished by atropine.

Kokin (1896), working in Pavlov's laboratory, showed that drops of secretion could be seen on the dog's exposed trachea when the appropriate nerve was stimulated, and interpreted this observation as showing that tracheal mucus secretion was under the influence of the nervous system.

Reflex Stimulation of Glands.

After a control period of 1 hour the central end of one cut vagus was stimulated, in one experiment, for 1 minute, with an interval of 1/2 minute, for 55 minutes. A considerable amount of mucus secretion, first apparent after 15 minutes' stimulation, appeared from an incision made in the trachea. In another experiment the nerve was stimulated for 1 2/5 seconds with a rest period of 1 second for 1 1/2 hours. At the end of 10 minutes mucus began to appear, and continued to collect as long as the stimulus was continued. It was necessary to take particular precautions to keep the vagus warm and moist for the success of the experiment. This experiment thus shows the existence of centripetal fibres in the vagus, stimulation of which causes reflex mucus secretion.

Effect of Pilocarpine.

The trachea of a decerebrated or anæsthetized cat was opened longitudinally, and bleeding stopped by a small cautery. It was then opened out to obtain a view of the mucosa. This was carefully dried with filter-paper and an intravenous injection of pilocarpine given. Secretion could then be seen collecting in droplets on the surface of the trachea. This action of pilocarpine was abolished by atropine.

The secretion by pilocarpine was also measured photographically (Fig. 7). This experiment revealed the motor effects of pilocarpine on the tracheal muscle. In Fig. 7 the sudden upward rise of the oil level can be seen. This was sufficient to carry it off the drum. After the administration of six doses of 0·25 c.c. of 1 in 1000 pilocarpine, 0·20 c.c. of 1 per cent. atropine was given, which caused the level of the oil to recede owing to relaxation of the trachea muscle. The difference of levels between that at the beginning and that at the end of the experiment represents the amount of mucus secreted in the time. Also, after pilocarpine, droplets can be seen collecting on the surface of the trachea. This appearance is best interpreted by supposing that the fluid is coming from the ducts of the buried glands.

From results obtained on other similar glands, *e. g.* the salivary, it may be concluded that pilocarpine stimulated the same glandular elements as did the nerve supply.

Attempts in the cat to see the appearance of droplets on the surface of the trachea in response to nerve stimulation have given somewhat equivocal results, as the observations are not easy to make unless a pronounced change occurs—as is the case with pilocarpine ; Kokin (1896), however, observed this to be the case in the dog.

Are the goblet-cells similarly innervated, or do the two sets of mucus-producing cells react in different ways ? It has been shown elsewhere that the goblet-cells of the colon are not under nervous control (Florey, 1930). It was hoped that the photographic method of recording would be of assistance in settling this and other points. It might be reasonably assumed that stimuli from the mucous membrane of the trachea would activate at least the buried glands reflexly—the efferent side of the arc clearly exists. This reflex mechanism would be inhibited by atropine. If the goblet-cells were similar

to those of the colon they would not be influenced by the drug. Accordingly olive oil containing mustard oil was substituted for pure oil in the tracheal preparation. It was hoped to obtain a curve of a certain steepness due to secretion from the glands, which could be inhibited by atropine, and so produce a lessening of the rapidity of ascent of the curve. Unfortunately the contractability of the trachea interfered with the experiment.

It was found that the placing of an irritant in the trachea caused a steady rise of oil level, the rapidity being greater the stronger the mustard-oil solution. This rise was quite uninfluenced by cutting both vagi or by a moderate dose of atropine. A very large dose slowed the rise, but did not abolish it. The rise was not caused by secretion, because if a rapid, considerable rise were produced, and the animal killed and examined, there was always a totally insufficient amount of secretion present to account for the volume change. The production of œdema in the mucosa by the irritant would not account for the alteration of oil level, for some tracings were obtained in which the oil level made an almost vertical ascent, *i. e.*, a considerable reaction occurred within a few seconds.

It was thought that mustard oil might be a specific stimulant for the smooth muscle, so a solution of ammonia in serum was substituted, with, however, the same results.

Nevertheless, it was found possible to show the reverse side of the picture. Immediately after anæsthetization a cat was given 0·2 c.c. of 1 per cent. atropine and the tracheal pouch filled with oil (3 drops mustard oil in 50 c.c. olive oil). The oil was left in for 3 hours, when the animal was killed and the pouch evacuated. A core of thick elastic mucus had been produced with a certain amount of slightly pink fluid. From the presence of the latter it might be inferred that considerable damage to the mucosa had occurred. This, however, only appeared somewhat reddened, while no petechial hæmorrhages or other signs of severe tissue damage could be seen. This experiment was repeated several times with larger (up to 1 c.c.) doses of atropine, and with the same result.

When atropine was omitted a similar product was obtained, though perhaps there was more of it, but the quantities could not be accurately measured.

Another method was adopted which gave a similar result. By means of a ball-valve arrangement the inspired air bubbled through a 1·5 per cent. solution of ammonia (ammon. fort.) before passing to the lungs of a cat through a tube inserted between the vocal cords. The cords gripped this tube and made a good joint. When both vagi were intact a plentiful secretion of mucus appeared in the trachea. Even after cutting the vagi and large doses of atropine, secretion was still produced, though whether this was less in amount was difficult to determine.

Still further evidence will be given later that copious mucus secretion can occur after thorough atropinization. Thus it will be seen that the foregoing methods have failed to give unequivocal evidence that stimulation of the tracheal mucosa reflexly excites the buried glands to secrete. However, all forms of experiment agree in showing that a very considerable secretion may occur after the cutting of both vagi and after thorough atropinization.

It has not been possible to illustrate a reflex effect on the glands from a

local stimulation by the utilization of the photographic method of recording tracheal secretion. But, on the other hand, it can be definitely concluded from its use that irritation of the lower part of the trachea does not cause secretion in the upper part, *i. e.*, that no long reflexes exist between the lower and upper portions.

By the use of histological methods the following observations bearing on the above experiments have been made. The usual technique consisted in fixation in "Susa" (Heidenhain, 1915; 1917), and staining in Delafield's hæmatoxylin followed by muci-carmine.

(1) *Nerve stimulation of tracheal glands.*—The control to these observations was furnished by excision and immediate fixation of a segment of trachea before stimulation was begun. The deep glands in the stimulated trachea showed the classical signs of exhaustion, viz., reduction in the amount of histologically demonstrable intra-cellular mucin; the localization of the latter (when present) in the tips of the cells; its presence, usually in abundance, in the acini and ducts.

(2) *Pilocarpine stimulation of tracheal glands.*—After repeated doses of this drug, changes similar, but culminating in an almost complete disappearance of the mucus were noted. (See fig. 2.)

(3) *Goblet-cells.*—Experiments in which the nerves were directly stimulated have not given histological evidence permitting of definite conclusions. Successful preparations were, however, obtained after administration of pilocarpine, the goblet-cell content being increased, as a preliminary, by a method to be described later. Discharge of the goblet-cells did not seem to be in excess of the normal, although the tracheal glands were almost completely exhausted.

(4) *The effects of application of mustard oil.*—Diluted mustard oil was applied to a tracheal pouch in the manner described. Histological evidence was obtained showing that (*a*) the tracheal glands showed exhaustion similar to that obtained after nerve-stimulation or pilocarpine. (*b*) Clear-cut results were not obtained in regard to the goblet-cells owing to the tendency to desquamation of the superficial epithelium. (*c*) In the heavily atropinized subject exhaustion of the deep glands was very evident. In these preparations numerous discharging goblet-cells were also observed. (*d*) It was noted, in an animal in which both recurrent and superior laryngeal nerves had been sectioned that the goblet-cells were discharging, exhaustion of the deep glands being also present.

Dog Experiments.

It was thought that perhaps the larger trachea of the dog would make observations on the expulsion of secretion from the gland ducts easier to observe.

The trachea of a large dog, anæsthetized with chloralose, was opened in the midline by means of a cautery. It was then opened out so that the inner surface could be observed. After trying various procedures calculated to produce a reflex secretion, the conclusion was reached that the method of visual observation was too hazardous for reliable conclusions to be drawn. The only unmistakable case in which secretion could be seen rising from the ducts was after the application of a faradic current to the mucosa, but as it was impossible

to separate satisfactorily a reflex effect from one directly acting on the nerves to the glands, it was decided to abandon this method of approach.

It would be reasonable to assume that the function of the glands is to react to some slight stimulus, *e.g.*, the presence of a foreign body in the trachea, so as to aid its expulsion, or that they are primarily concerned in keeping the trachea moist—a function similar to that of the glands of the mouth.

The following experiments were done on the assumption that such would be the case.

Cats prepared with tracheal pouches and two cannulæ as already explained were used. By means of either a water-suction pump or an electric blower, a stream of air was passed over the mucous surface of the trachea. This stream was first thoroughly dried. The most satisfactory arrangement was to pass the air over $CaCl_2$, firstly, and finish the drying over P_2O_5. After passing this perfectly dry air over the trachea, the moisture it took up therefrom was collected either in weighed $CaCl_2$ tubes, or, in what proved to be better, in tubes containing P_2O_5. It was thus possible to find the amount of water given off during stated time-intervals.

If the glands therefore were stimulated to produce fluid this would be evaporated, and the amount of water collected would be a measure of the amount of secretion poured out. If atropine were given (which paralyses the influence of the nerves on the deep glands), then the amount of water collected in unit time should show a decided fall, providing the protection of the tracheal surface against desiccation depended largely on the buried glands.

The surprising result was consistently obtained that the amount of water collected showed a very slight or no diminution after the administration of large doses of atropine.

For instance, dry air blown through trachea for 3 minutes and then P_2O_5 tubes attached to outlet :

Results : 1st 5 mins. weight water collected, 0·104 gm.
2nd 5 ,, ,, ,, ,, 0·130 ,,
3rd 5 ,, ,, ,, ,, 0·100 ,,

0·5 c.c. of 1 per cent. solution atropine sulphate given intravenously immediately.

Interval of 1 minute :

Results : 4th 5 mins. weight water collected, 0·109 gm.
5th 5 ,, ,, ,, ,, 0·113 ,,
6th 5 ,, ,, ,, ,, 0·202 ,,

The trachea was opened immediately and found to be dry. Air had been passed at the rate of 1 litre in 46 secs.

The following longer experiment indicated the steady rate at which the trachea can give off water.

1 hour { 1st 15 mins.; water caught, 0·243 gm.
2nd ,, ,, ,, 0·251 ,,
3rd ,, ,, ,, 0·261 ,,
4th ,, ,, ,, 0·266 ,,

2 hours	5th	15 mins.;	water	caught,	0·283 gm.
	6th	,,	,,	,,	0·265 ,,
	7th	,,	,,	,,	0·252 ,,
	8th	,,	,,	,,	0·260 ,,
3 hours	9th	,,	,,	,,	0·266 ,,
	10th	,,	,,	,,	0·248 ,,
	11th	,,	,,	,,	0·262 ,,
	12th	,,	,,	,,	0·268 ,,

When examined the trachea was covered with a small amount of mucus secretion, except for one small patch which appeared dry. The rate at which the air passed through was not measured.

The following experiment shows a very small drop following the administration of atropine:

	1st	15 mins.;	water	caught,	0·235 gm.
	2nd	,,	,,	,,	0·255 ,,
	3rd	,,	,,	,,	0·232 ,,
	4th	,,	,,	,,	0·233 ,,
0·3 c.c. atropine 1 per cent. given:					
	5th	15 mins.;	water	caught,	0·232 gm.
	6th	,,	,,	,,	0·193 ,,
	7th	,,	,,	,,	0·209 ,,
	8th	,,	,,	,,	0·210 ,,
	9th	,,	,,	,,	0·200 ,,
	10th	,,	,,	,,	0·218 ,,
	11th	,,	,,	,,	0·205 ,,
	12th	,,	,,	,,	0·204 ,,

On examination the trachea was covered with a moist coat, except for one small patch which appeared dry.

Further observations were made by similar methods. If the dried air were run over the trachea at the rate of 1 litre in 41 seconds, at the end of 1/2 hour, if not sooner, the trachea of an atropinized cat appeared dry. If, however, the cat were left for 1 hour after the cessation of the passage of the air and then examined, it was found that the trachea was covered by a thick coating of moist mucus. Clearly this abundant mucus secretion had gone on in spite of the atropine.

Histological examination.—The prolonged passage of dry air was found to be definitely harmful to the epithelium, desquamation being elicited. Such goblet-cells as were still *in situ* were found to be actively discharging. The tracheal glands were swollen, this being due to swelling of the constituent cells as well as to dilatation of the acini. The mucin content of the cells was increased. The submucosa was œdematous and its blood-vessels dilated.

It would thus appear that the air blast causes an acute inflammation, the bulk of the water collected, however, being the product of vascular transudation rather than secretion from the glands.

A modification of the above experiment was also used. The trachea of a cat was incised in the midline by means of a cautery and opened for inspection.

EXPLANATION OF FIGURES.

All figures drawn with camera lucida at a magnification of 455 diameters. Present magnification, × 365, owing to reduction. All figures on these plates are from sections of trachea of cat (except No. 3). Fixation in "Susa"; Delafield's hæmatoxylin and muci-carmine. B., Blood-vessels in submucosa; C., columnar epithelial cell; D., duct of tracheal gland; G., goblet-cell; M., mucus cell; P., polymorph leucocyte; S., serous cell; X, see explanation of figure in question.

FIG. 1.—Normal trachea showing goblet-cells containing mucus, and two in process of discharging it. A few of the serous cells contain intra-cellular mucus (X), which is present in all the cells of the mucous alveolus depicted.

FIG. 2.—After injection of pilocarpine. The mucus of the goblet-cells stains faintly, and is not appreciably reduced in amount; the gland cells are completely exhausted (except at X); some mucus in their lumina.

FIG. 3.—From a case of chronic human bronchitis. Note the almost complete substitution of large goblet-cells in place of the columnar elements; the former all contain histochemically detectable mucus and most of them are actively discharging it.

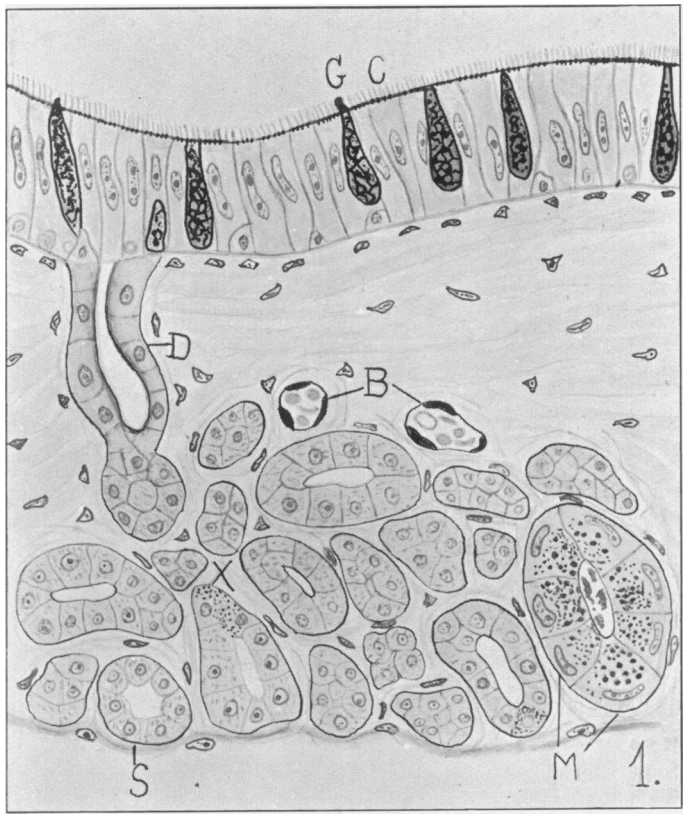
FIG. 4.—Intra-tracheal injections of 3 c.c. daily with 1/8 per cent. formol for 9 days. This has provoked a very marked increase in the number of goblet-cells; they also tend to be unduly large, and contain mucus, which is being discharged. The deep glands, here "serous" morphologically, contain intra-cellular mucus (X), but show no signs either of discharge or exhaustion.

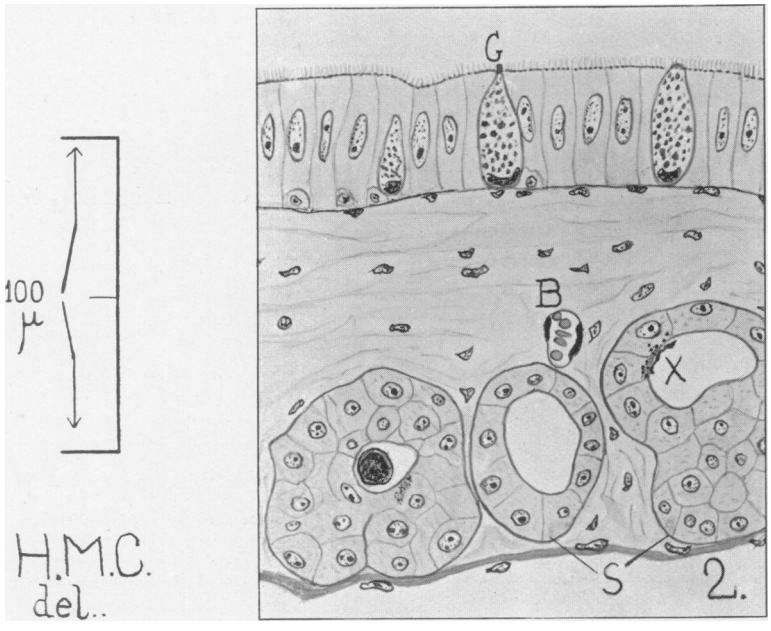
FIG. 5.—Daily intra-tracheal injections of 1/4 per cent. formol (of 5 c.c. each) for 7 days. Owing, presumably, to the stronger concentration used, the columnar epithelium has been replaced by one of the stratified type. It is rather similar to the transitional epithelium of the urinary bladder. Mitoses (X) are abundant. The deep glands contain no mucus and are clearly exhausted; there is a little in the alveoli and ducts.

FIG. 6.—Photographic record of secretion produced by alternate stimulation of both vagi. The rise in the curve is due to the accumulation of mucus, as there is no fall after the administration of atropine, which relaxes muscular contraction. There was no further secretion of mucus on stimulation after the dose of atropine. The horizontal lines represent a volume of 0·01 c.c.

FIG. 7.—Effect of administering successive doses of 1 : 1000 pilocarpine. There is a rapid muscular contraction which carried the record off the sensitive paper. On the administration of atropine there was a rapid relaxation of the contracted muscle. The difference between the level at the beginning and after atropine administration represents the amount of mucus secreted in the time. For purposes of reproduction the curve has been outlined in Indian ink and the photograph bleached.

FIG. 8.—*a*, Low-power photograph of a "patch" of small intestine removed at biopsy after being *in situ* 2 years. Note absence of villi, but very long crypts of Lieberkühn. The black substance beginning in the tips of the cells towards the bottom of the crypts and gradually increasing towards the surface is mucus stained red by muci-carmine. *b*, High-power photograph of "patch" stained by Masson's aniline blue-ponceau-acid fuchsin. This is a portion of the patch showing the enormous increase of the number of goblet-cells present.





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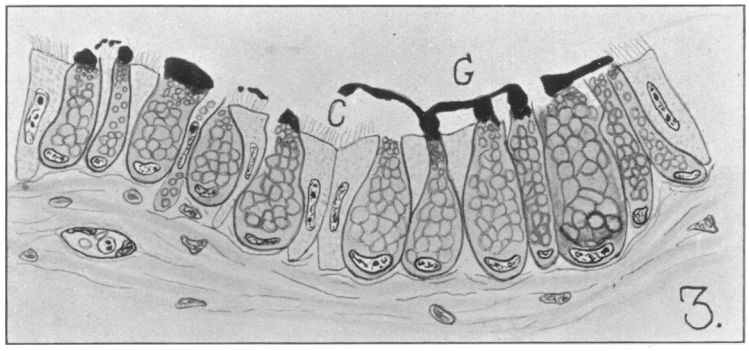

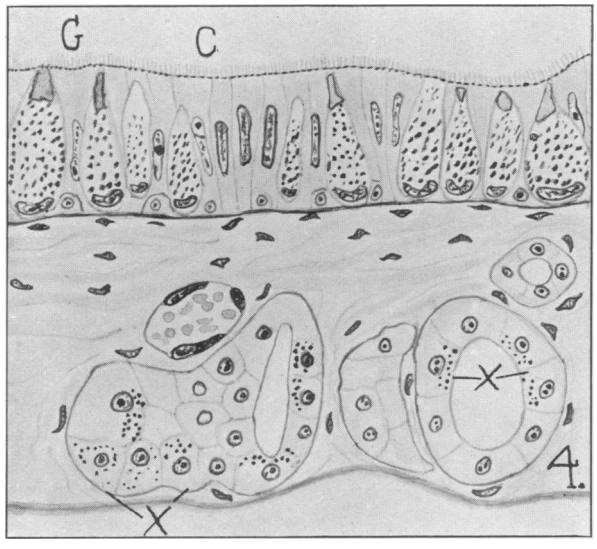

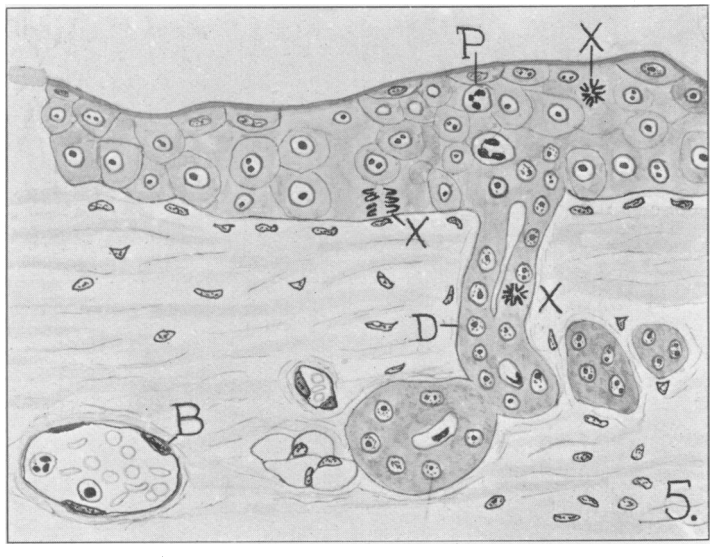

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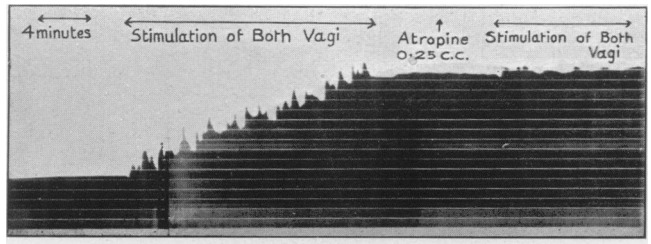

FIG. 6.

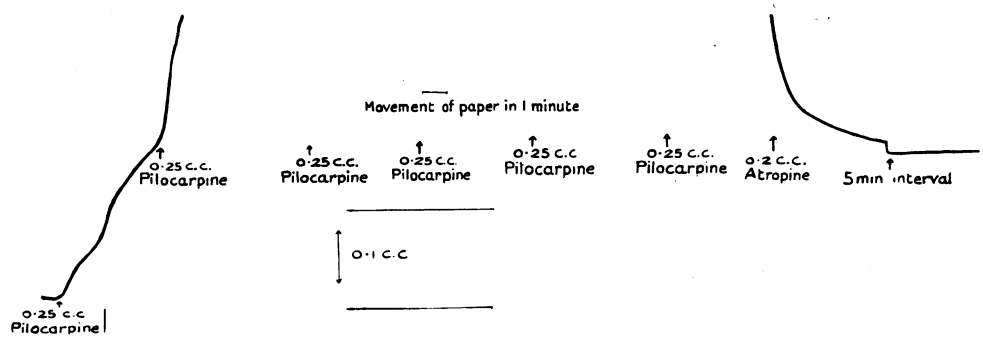

FIG. 7.

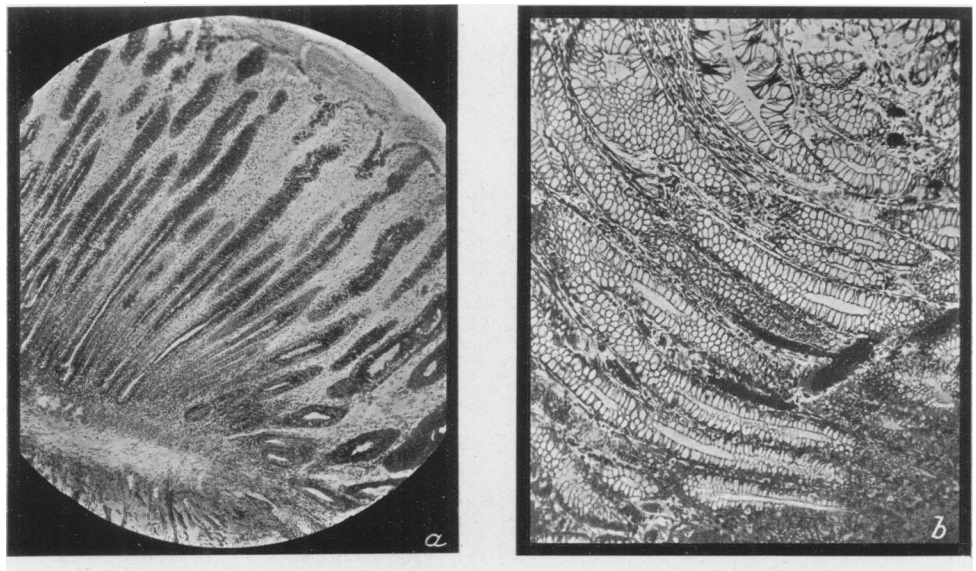

FIG. 8.

A concentrated beam from a " pointolite " was so adjusted that a bright reflection was obtained from a portion of the moist mucosa. By directing a blast of dry air against this spot the moisture was rapidly removed and the mucosa then presented a matt surface. The time was then taken by a stop-watch for the glistening appearance to return.

Atropine was then given intravenously and even applied locally and the time again noted.

The following figures were obtained :

Intervals for reappearance of glistening surface, 8 secs., 9 secs., 9 secs.

0·5 c.c. 1 per cent. atropine injected intravenously :

After 5 minutes' interval : 8 secs., 9 secs., 8 secs.

Clearly no difference.

A further modification of this type of experiment was as follows : A slow stream of dry air was passed through a tracheal pouch, which at the end of an hour was excised and examined. The mucosa was moist, but there was no free fluid or mucus. In other cats the air was bubbled through ether before being dried. At the end of an hour there was an abundant collection of free fluid containing mucus inside the pouch. The experiment was then repeated, giving as a preliminary 0·4 c.c. of 1 per cent. atropine intravenously, but there was still an abundant collection of rather thin mucoid fluid. The administration of the atropine had made no appreciable difference to the amount of fluid present. The fluid produced by the ether irritation was much thinner and more watery than that produced by other forms of irritants tried. It was quite clear, and showed no macroscopical evidence of being produced by gross damage to the mucosa. Histological examination of specimens from these experiments gave the following results :

Normal trachea.—There were goblet-cells in moderate numbers—mostly faintly positive with muci-carmine. The glands showed a fair proportion of cells containing mucin. There was a little positive secretion, some of it in the ducts, many of which were dilated.

Trachea treated with ether.—The general appearance is much the same as the above, but the proportion of positively stained gland-cells is apparently smaller than in the normal. There is so great a regional variation in the distribution of the goblet-cells that it is difficult to make any comparison between this and the normal specimen. In some places there is a thick layer of material, negative for mucin, lying against the epithelium. It contains some oval bodies which stain like mucus, and which might be the contents of goblet-cells still retaining the shape of the cells.

In another experiment, 0·4 c.c. of 1 per cent. atropine was given intravenously and the animal killed at the end of 40 minutes.

Normal trachea.—General condition similar to that of the preceding experiment.

Trachea treated with ether.—Condition as in control trachea.

In another similar experiment the trachea treated with ether showed a fair number of feebly positive goblet-cells, the gland-cells being mostly negative, their lumen dilated.

Thus, histologically, although a very copious secretion is called forth by ether, there is no very good evidence to show that the goblet-cells are emptied.

The thin secretion noted macroscopically from its staining reactions does not appear to contain much mucus. Inasmuch as this fluid appears whether the deep glands are paralysed by atropine or not, it would seem that it must come largely from the tracheal surface, though the goblet-cells do not appear to be particularly involved. On the other hand, some histological evidence was obtained that the deep glands secrete when stimulated by the ether, but that this is inhibited by atropine.

Support for this view can also be obtained from observations on an opened trachea dried by an air-blast after thorough atropinization. After drying, the surface rapidly becomes moist again, but this fluid is " thin," and does not appear to be very mucoid—a fact better observed microscopically in the way to be described.

Microscopical Observations in vivo.

The trachea of a cat was opened in the midline by a hot knife. This was found to cause a minimum of damage, and saved considerable trouble in stopping bleeding from cut vessels after a scissors cut. The trachea was held apart sufficiently for observation by small hooks. The source of light came from a 100 candle-power " pointolite," while a binocular dissecting microscope was used for observation. When observed with a magnification of approximately 25 diameters the blood supply of the mucosa was readily seen. Where the light was sharply reflected from the tracheal surface the activity of the cilia could be clearly observed. Where the light was more diffusely reflected, what seemed under the microscope to be a " river " of secretion could be seen rapidly passing along the trachea towards the larynx. This constantly moving layer made it impossible to distinguish where the secretion was coming from.

Various procedures were adopted to see if this could be determined. A blast of dry air was directed on to the trachea till it appeared quite dry and matt. On removal of the blast the mucosa rapidly swelled again and the surface became moist. This procedure was repeated many times, but it was never possible to separate clearly the contribution of the tracheal surface and the deep glands to this moisture. The moisture appeared at first to come evenly from all the surface. After the lapse of some time large oval collections on the general moist surface appeared. These perhaps represented secretion issuing from the deep glands. A point previously mentioned as to the rather watery character of this secretion could be well appreciated by touching the secretion with a needle—it did not adhere. Mucoid secretion, such as was normally present before interference, or after pilocarpine, could be pulled into strings. After atropine no clear-cut differences were detectable, the rather watery secretion appearing as before. It might be thought that these short blasts of air inflicted severe damage on the mucosa, but such did not appear to be the case. When dried the ciliary action could be seen to cease, but with the return of the moisture it once more appeared. It was only after repeated dryings that the cilia permanently ceased to function.

When a small drop of suspension of graphite ink (hydrokollag 300) in normal saline was applied to the trachea, the following picture of the clearing action of the cilia was very striking: The graphite particles adhere to the strands of mucus as they are being propelled along the trachea. Some of the particles

sink down and tend to remain, or at least are rather slowly moved forward until they are caught on the strands of mucus. These latter tend to be rolled up as they pass along, the process being assisted by the elasticity of the mucus. The strands appear to stick in some portion of their length from time to time, while the more forward portions are propelled onwards by the cilia. The force of the latter is sufficient to rupture the mucus strands, which then tend to roll up owing to their elastic recoil.

As Leonard Hill (1928) has shown on excised tracheæ, this power to remove small foreign bodies is very marked. From our observations the ability would appear to depend to a certain extent on the " stickiness " of the mucus. Another point which emerged was that when a considerable quantity of mucus secretion was present, the mucus stream moved more quickly than when the normal " resting " secretion alone was present. For instance, after a pilocarpine injection, the rate of the passage of the mucus stream was considerably accelerated.

Another attempt was made to utilize the above observations to demonstrate the reflex activity of the deep glands. If a dry substance is placed on the mucosa this has first to be thoroughly moistened by secretion before it is removed by the cilia. For these observations fine starch powder was found to be most suitable, though animal charcoal gave the same results.

The following figures were obtained in such an experiment. A piece of trachea only 3 cm. long was used, the portion below being isolated by destroying an intervening strip of mucosa. Times from the application of the starch particles to the first move were taken by stop-watch, the observation being made with the binocular microscope. A, B, etc., represent separate applications of starch :

	A.	B.	C.	D.	E.	
Minutes	2′ 30″	3′	2′ 30″	2′	2′	First move noted.
	6′	4′ 45″	3′	5′	3′ 45″	All particles moving.

1 c.c. of atropine was then given and 10 minutes allowed to elapse :

	F.	G.	
Minutes	3′	2′ 30″.	Started to move.
	4′	4′ .	All particles moving.

In this experiment a particle was observed to travel 3·5 cm. in 1 minute. This experiment was repeated with the same results, *i. e.* the trachea was found to be able to move dry particles equally well whether the deep glands were atropinized or not.

It was observed in some experiments that after atropine there appeared to be a decrease in the amount of secretion passing across a field though there was still an adequate flow. (Care was taken to see that the flow only came from the portion of trachea observed by destroying the mucosa below it, either with the cautery or by scraping with the scalpel.)

An attempt was made to make this observation quantitative by catching the stream of mucus at the end of its journey. Small pieces of blotting-paper about $1\frac{1}{2}$ mm. wide were pressed on to the space extending between the posterior fold and the cut edge of an opened trachea, to collect the secretion

forced up from about 3 cm. of trachea. These pieces were replaced from time to time, the thick mucus collected against the edge of the blotting-paper being assisted on to it by a fine glass rod.

These pieces were weighed before and after the experiment in covered watch-glasses to avoid evaporation.

Protocol of such an Experiment.

1st watch-glass in 15 mins, ·0075 gm.
Animal then given 1 c.c. 1% atropine intravenously.
20-minute interval :
2nd watch-glass in 15 mins., ·0055 gm.
3rd ,, ,, ·0014 ,,

There is a distinct drop in the amount of secretion, but the quantities collected are very small. This small amount of secretion gives the impression microscopically of a moving layer of mucus of some considerable thickness.

An attempt to measure the secretion was made by applying a small piece of blotting-paper to the trachea, and noting the time taken for it to appear uniformly moist. It was thought that if the deep glands were contributing any considerable amount of secretion under these conditions, then, on giving atropine, it should take longer for the paper to get moist. Care was taken to shield the piece of paper from the current of secretion constantly travelling up the trachea.

Results of an Experiment.

Times taken for paper to become completely moist (each a separate application) :

1′ ; 2′ ; 2′ 50″ ; 2′ 30″ ; 2′ 50″ ; 2′ 30″ ; 2′ 30″ ; 2′ 30″ ;
1 c.c. of 1% atropine given :
3′ ; 2′ ; 2′ 30″.

Here again, therefore, the contribution of the deep glands did not seem to be very great.

That, however, the deep glands are constantly contributing to the tracheal secretion was seen in some experiments with the use of graphite ink.

Thin streams of mucus issuing from a single point could be seen to stand out against the dark background of the ink, much in the same way as spirochætes do in an Indian ink preparation. These streams were not observed after atropine.

After a dose of pilocarpine large eruptions of a similar nature were observed, especially if the cilia were first killed by some bromine vapour.

These observations could not be satisfactorily extended owing to the difficulty in controlling the conditions so as to keep them constant over a period of time.

It is thus abundantly clear from these experiments that the surface of the trachea can become moist very easily when subjected to any stimulus employed, without the participation of the deep glands. Nevertheless, it must be admitted that any stimulus which it has been practicable to use is probably more vigorous

than those to which the trachea is normally exposed, approximating more to pathological conditions such as a bacterial tracheitis.

DISCUSSION.

It appears certain from the physiological and histological results taken in conjunction, that stimulation of the appropriate nerves activates the tracheal glands. Exhaustion of these can, in fact, be brought about by sufficiently prolonged stimulation. This contention is strongly supported by the results of pilocarpine injections, which produce considerable secretion, followed by microscopically demonstrable exhaustion of the glands. The secretion caused by both these methods is inhibited by atropine.

No satisfactory evidence has been forthcoming that the goblet-cells can be discharged by nerve-stimulation. Even the administration of pilocarpine in doses sufficient to exhaust the tracheal glands leaves the goblet-cells not appreciably affected. Attempts by several methods to demonstrate the reflex activation of the tracheal glands have not furnished any conclusive evidence, but direct stimulation of the central end of the vagus will cause secretion.

The application of dilute mustard oil has been shown to cause microscopically demonstrable exhaustion in animals (*a*) with all nerves intact, (*b*) with both the recurrent and superior laryngeal nerves sectioned, and (*c*) after the administration of massive doses of atropine. We therefore conclude from these experiments that activity of the tracheal glands is due to direct action of the irritant upon the gland-cells. Some evidence was obtained that the goblet-cells were partly discharged by the mustard oil, though complete exhaustion was never noted.

The passage of dry air caused inflammation and the production of considerable amounts of fluid, which, however, did not seem to be formed by glandular activity. This fluid appears to come directly from the vessels.

Experiments in which dry ether vapour was passed slowly through the trachea were found to produce less irritation than dry air passed more rapidly. Here some microscopical evidence was obtained of the inhibition of the tracheal glands by atropine.

A striking feature of our observations is the ease with which the surface of the trachea can produce fluid without any significant intervention on the part of the glands. It is to this that we attribute our inability to demonstrate satisfactorily reflex activation of the glands from stimulation of the tracheal surface. We consider, for this reason, that experiments such as those performed by Rossbach (1882) and Calvert (1896) are of limited value, in that the open trachea was first dried with blotting-paper and the time to get wet again noted. Rossbach and Aschenbrandt (1881) noted that, after section of the nerves and clamping of the trachea in two places, the degree of secretion remained unaltered. They concluded that little secretory activity (if any) was elicited through the nervous channel. This inference seems to be quite unjustified owing to the experimental difficulties which we have indicated.

A point of histological interest is that the hard and sharp division of gland-cells into mucous and serous elements would appear to be open to doubt. Thus, cells and acini, which on a purely morphological basis would be called

serous, proved, after treatment with specific stains for mucus, to contain this substance. Actual discharge was also noted.

THE EFFECT OF CHRONIC IRRITATION ON THE NUMBER OF GOBLET-CELLS.

Human bronchitis.—Cases of this were investigated. Sections were made from 14 of these and stained by the modification of muci-carmine described below. This was essential, as application of the ordinary method gave very poor results. Post-mortem change may possibly account for this.

Sections were mordanted for 10 minutes in a 0·5 per cent. solution of aluminium chloride in 50 per cent. alcohol. They were then stained for 1 hour in a 1 in 3 to 1 in 4 solution of muci-carmine in tap-water.

Of the 14 bronchi examined the epithelium was lacking in 5 ; in some cases this was due to acute inflammation having made it slough ; in one or two, post-mortem desquamation appears to be the cause.

In one specimen there was no definite increase in the goblet-cells, though the deep glands were undoubtedly highly active.

In the remaining 8 the goblet-cells were greatly increased in numbers. In 2 specimens the major part of the mucosa was constituted solely of these cells (see Fig. 3). Many were in process of extruding a plug of mucus. The deep glands showed dilated alveoli, the cells and lumina of which contained strongly positive mucus. The majority of these cells were mucous in type, but elements which, on a purely morphological basis, would be unhesitatingly termed " serous," contained a secretion, usually granular, which readily stained with the specific mucin stain.

One curious feature remains to be noted : A number of the columnar cells of the ducts contained mucus and appeared to be contributing it to the general production.

To sum up : In all cases (except one) in which the mucosa was sufficiently intact for estimation to be made, a widespread substitution of goblet-cells for columnar cells was obvious. So also was the great activity of the deep glands. The scarcity of " serous " cells in many of these sections, when taken in conjunction with the frequent presence of mucus in these elements, suggests that they are capable of elaborating mucin.

Thus in bronchitis, not only is there evidence of greatly increased production of mucus by the buried glands, but the ciliated surface epithelium itself may become almost totally replaced by cells of the goblet type.*

The effects of intratracheal injections of formalin were investigated. The concentrations used were 1/4 and 1/8 per cent., the duration varying from five to fourteen daily injections of 3 c.c. each. The technique was as follows : The trachea was injected under deep ether anæsthesia. A good view of the glottis was obtainable by opening the jaw with the animal supine and the head fully extended. A long glass tube, to which a syringe was attached, was then passed through the glottis for a distance of 3 cm. The animal was held head up while the formalin was forcibly injected.

* We are indebted to Dr. W. D. Newcomb, of St. Mary's Hospital, and also to Dr. Baker, of the Middlesex Hospital, for these specimens.

Two main effects were noted : firstly, the epithelial cells (Fig. 5) lost their columnar shape and cilia, and became transformed into polygonal elements rather similar to the transitional epithelial cells of the urinary bladder. (This was particularly noticeable after injections of 1/4 per cent. formalin.)

Secondly, there was an enormous increase in the number of goblet-cells as compared with normal cats (Fig. 4). In some sections, indeed, they greatly outnumbered the columnar cells. From the fact that many of these cells are deeply situated and abut on the basal layer, we conclude that they are probably derived from the undifferentiated cells of this region.

The glands generally showed much mucin, both in the cells and in the lumina, though in some cases there was evidence that their enhanced activity had resulted in exhaustion.

Observations on the bronchi of guinea-pigs subjected to dust inhalation (Carleton, 1924 ; 1927) also revealed an increase in the number of goblet-cells. In cases where the bronchitis was marked, not only is the epithelium largely composed of these elements, but large numbers of them lie in the plugs of *débris* in the lumina of the bronchi.

Chronic irritation of the small intestine.—A " patch " of ileum was inserted on to the surface of the abdominal wall in a dog with the same technique as previously adopted for the colon (Drury, Florey and Florey, 1929). A lateral anastomosis was used. Biopsy of the mucosa of the patch was made two years after the insertion of this patch. Microscopically it was noted that the villi had disappeared, the mucosa consisting solely of deep crypts. Concomitantly with this the number of goblet-cells was enormously increased (Fig. 8). These changes are attributable to chronic irritation induced by exposure of the mucosa, as evidenced by a slight leucocytic infiltration.

It would thus appear, from the four types of observations quoted, that chronic irritation of a mucous membrane normally provided with goblet-cells results in a striking increase of these elements.

SUMMARY.

1. Evidence has been produced to show that the glands of the trachea are activated by stimulation of the recurrent laryngeal nerves.

2. Pilocarpine causes similar activity.

3. The secretion provoked by both 1 and 2 is inhibited by the administration of atropine.

4. Stimulation of the central end of the vagus causes these glands to secrete reflexly.

5. Owing to technical difficulties we have been unable to obtain satisfactory evidence of reflex secretion caused by direct stimulation of the mucosa. We regard this, however, as a probability.

6. The immediate effects of various types of irritant are described.

7. Chronic irritation of mucosæ such as those of the trachea, bronchi and small intestine, leads to a great increase in the number of goblet-cells. In the case of the trachea more intense irritation causes the columnar and goblet-cells to be replaced by a stratified epithelium rather similar to that of the urinary bladder.

8. Gland-cells which on a morphological basis would be termed " serous " appear often to be capable of elaborating mucus, as evidenced by their histochemical reactions.

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STUDIES IN *B. PESTIS* ANTIGENS: I. THE ANTIGENS AND IMMUNITY REACTIONS OF *B. PESTIS*.

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MANY years ago Rowland (1914) made the observation that *B. pestis*, when grown at 37° C., developed a gelatinous envelope which he demonstrated by means of film preparations in indian ink.

A tiny loopful of agar culture or of the centrifuged deposit of a broth culture, grown at 37° C., is mixed with an equally small loopful of suitable indian ink so that the resulting film shall be a thin one, and is examined under a coverslip by the oil-immersion lens. Each plague germ is seen to be surrounded by a clear zone of considerable extent—the gelatinous envelope. Similar preparations made from cultures grown at 20° C. show no envelopes; the oscillating particles of indian ink directly bombard the naked bodies of the bacteria. At 26° C. there will be a meagre development in the case of virulent passage strains, while with old non-virulent laboratory strains it is not until a still higher temperature is reached that envelopes begin to appear—a fact which is of interest when correlated with the lack of prophylactic value possessed by non-virulent cultures. At 37° C., however, the phenomenon is, in the author's experience, a constant one; as, at this temperature, envelopes develop upon